SUPPRESSION OF A PLEIOTROPIC MUTANT AFFECTING GLYCEROL DISSIMILATION

Myra Berman, Noe Zwaig*, and E. C. C. Lin

Department of Bacteriology and Immunology

Harvard Medical School, Boston, Massachusetts

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A mutant of Escherichia coli which lacks Enzyme I of the phosphoenolpyruvate phosphotransferase system and can not grow on glycerol was studied. The inability to utilize glycerol can be suppressed in two ways: 1) conversion of the L-α-glycerophosphate system from inducibility to constitutivity, and 2) addition of cyclic AMP to the growth medium. The growth defect of such a mutant on glycerol is therefore attributable to failure of induction of the glycerol enzymes.

A bacterial phosphotransferase system catalyzes the phosphorylation of several sugars by the following reactions (1):

$$\text{PEP}^{**} + \text{HPr} \xrightarrow{\text{Enzyme I}} \text{P-HPr} + \text{pyruvate}$$

$$\text{P-HPr} + \text{sugar} \xrightarrow{\text{Enzyme II}} \text{Sugar-P} + \text{HPr}$$

Both Enzyme I and HPr appear to be unique and serve a family of Enzymes II (2). Mutational loss of either Enzyme I or HPr has pleiotropic effects, whereas the loss of an Enzyme II in general affects the dissimilation of only one sugar (3, 4, 5).

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* Present address: Escuela de Biologia, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela

** Abbreviations used: PEP, phosphoenolpyruvate; HPr, a histidine-containing protein of low molecular weight; P-HPr, the phosphorylated protein; L-α-GP, L-α-glycerophosphate; glp, the L-α-GP regulon; cyclic AMP, 3', 5' adenosine monophosphate; lac, the lactose operon.

Enzyme I mutants in E. coli fall into two classes: a) those which can not grow on the sugars which are known to be phosphorylated by the PEP system, such as glucose, fructose, mannose, mannitol, and sorbitol (3) and b) those which, in addition, fail to utilize several or all of the following compounds: glycerol, succinate, maltose, lactose, and galactose (6). The catabolism of these substrates is not known to be directly connected with the PEP system. Since this laboratory has been engaged in the study of the control and utilization of glycerol (the glp system), we decided to investigate the relationship between Enzyme I loss and the failure to grow on glycerol.

An ATP-dependent kinase is obligatory for the dissimilation of glycerol in E. coli (7). The product of the reaction, L-α-GP, is converted to triosephosphate by a dehydrogenase (8). Exogenous L-α-GP (the true inducer) can also be taken up by the cells through the mediation of an active transport system (8, 9). To determine whether the failure of a mutant lacking Enzyme I (strain 223) to dissimilate glycerol reflects an alteration in the synthesis of the kinase or the dehydrogenase, we grew these cells and those of the wild-type parent (strain 11) on pyruvate mineral medium and pyruvate plus glycerol or DL-α-GP. As shown in Table I, the mutant formed much less glycerol kinase and L-α-GP dehydrogenase than did the wild-type, irrespective of whether glycerol or L-α-GP was added as inducer. It would therefore appear that the Enzyme I lesion interferes with the induction mechanism in the mutant. To test this supposition we obtained a strain (227) which, in addition to the Enzyme I mutation, carries a glp R^c mutation. Such a mutant was found to grow on glycerol (Fig. 1) but still could not metabolize

Table I. Enzyme levels of the glp system in wild-type (strain 11) and mutant (strain 223) cells.

	Strain 11		Strain 223	
Inducer	Glycerol kinase*	L-α-GP dehydrogenase*	Glycerol kinase	L-α-GP dehydrogenase
None	0.07	< 0.005	0.02	< 0.005
Glycerol	1.0	0.028	0.04	< 0.005
DL-α-GP	0.85	0.041	0.09	< 0.005

Strain 11, with wild-type phosphotransferase system, is a derivative of E. coli K 12, AB 313 (10) [from Dr. A. L. Taylor] which grows faster on mannitol than its parent. Strain 223 was isolated from strain 11 as a mannitol negative clone on eosin methylene blue indicator plates after mutagenesis with ethyl methanesulfonate (11) and penicillin selection (12). The mutant cells contained less than 1% of the Enzyme I activity found in the wild-type cells (13). For the above experiments cells were grown on pyruvate (0.02 M) mineral medium (13) with glycerol (0.02 M) or DL-α-GP (0.04 M) added as inducer for several generations and were harvested at a density of 4 x 10^8 cells/ml. The glp enzymes were assayed as previously described (7).

* μmoles of product formed/min/mg of protein

other carbon sources, such as mannitol and succinate.

Recently Pastan and collaborators have shown that a pleiotropic carbohydrate mutant of E. coli lacking Enzyme I which does not grow on lactose can be made to grow on this compound if cyclic AMP is added to the medium (14). In view of their finding and of the fact that constitutivity of the glp system suppresses the genetic effect of the Enzyme I mutation on glycerol utilization, we tried to induce the glycerol enzymes in strain 223 by adding cyclic AMP to cells growing on pyruvate.

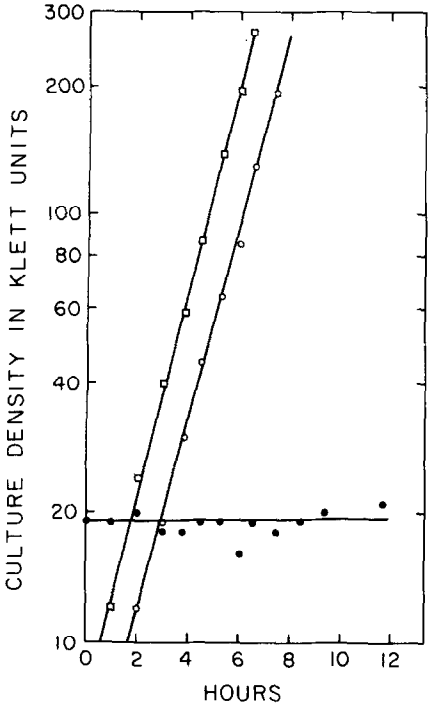

Figure 1. Growth responses of strains 11, 223, and 227 in a glycerol medium.

Cells of strain 11 (wild-type -o-), 223 (Enzyme I negative -●-), and 227 (Enzyme I negative and glp constitutive -■-) were grown on 0.2% arabinose, washed, and transferred to a glycerol medium (0.02 M). Strain 227 was constructed as follows: cells of strain 223 (Hfr, leu$^-$, his$^+$, glp R$^+$) were mated with strain 177 (F$^-$, leu$^+$, his$^-$, glp R^c) and spread on arabinose plates. A clone lacking Enzyme I and constitutive in the glp system was identified by enzyme assays.

The results in Table II show that cyclic AMP permitted the glycerol kinase in the mutant to be induced to almost wild-type levels, a 17-fold increase. Furthermore, we found that the addition of cyclic AMP to the culture medium also enabled the mutant to grow on glycerol (Fig. 2).

The study of Pastan and Perlman on the lac system and the results reported here on the glp system suggest that Enzyme I

Table II. Effect of cyclic AMP on the inducibility of glycerol kinase in wild-type (strain 11) and mutant (strain 223) cells.

Addition to medium	Glycerol kinase activity*	
	Strain 11	Strain 223
DL-α-GP	0.39	0.03
DL-α-GP + cyclic AMP	0.91	0.50

Duplicate cultures of each strain were grown on pyruvate (0.02 M) to a density of 4×10^8 cells/ml, at which point DL-α-GP (0.04 M) was added to all four cultures and cyclic AMP (5mM) was added to one of the duplicates. The cells were harvested 60 minutes after the additions and assayed for the kinase.

* μmoles product formed/min/mg

mutants of class b could also be made to grow on succinate, maltose, and galactose if cyclic AMP were added to the medium, or if their respective systems were converted to constitutivity. In addition, there may be other catabolic systems whose inducibility is impaired by the mutations affecting Enzyme I. Indeed we found that the induction of the tryptophanase system was also defective in strain 223 and that cyclic AMP overcame this defect.

It should be stressed that although the defective metabolism of substrates peculiar to class b mutants may be suppressed in two ways, genetically by constitutivity and phenotypically by cyclic AMP, the connection between the control mechanisms of the affected pathways and the phosphoenolpyruvate system is not yet clear.

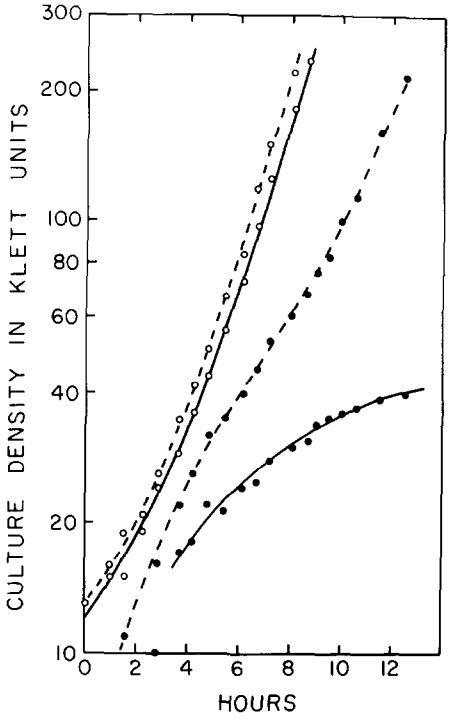

Figure 2. Restoration of growth on glycerol of strain 223 by cyclic AMP.

Cells of strain 11 (o) and strain 223 (●) were grown on 0.02 M pyruvate and transferred to a mineral medium containing 0.02 M glycerol with (---) and without (——) cyclic AMP (5mM).

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